FEBS Letters 587 (2013) 3008–3013

FEBS Letters

journal homepage: www.FEBSLetters.org

The essential gene *YMR134W* from *Saccharomyces cerevisiae* is important for appropriate mitochondrial iron utilization and the ergosterol biosynthetic pathway

Gabriel Moretti-Almeida [a], Luis E.S. Netto [b], Gisele Monteiro [a,*]

[a] Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo – USP, Brazil
[b] Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo – USP, Brazil

ARTICLE INFO

Article history:
Received 17 May 2013
Revised 4 July 2013
Accepted 9 July 2013
Available online 23 July 2013

Edited by Francesc Posas

Keywords:
Ergosterol biosynthesis
Iron homeostasis
Mitochondrial function
Antioxidant response
Essential gene
Uncharacterized gene

ABSTRACT

A thermosensitive strain (*YMR134W*^{ts}) of the essential gene *YMR134W* presented up to 40% less ergosterol, threefold lower oxygen consumption and impaired growth on respiratory conditions. The iron content in the mitochondrial fraction of *YMR134W*^{ts} cells was considerably low, despite these cells uptake and accumulate more iron from the culture media than wild-type cells. *YMR134W*^{ts} cells were also more susceptible to oxidative stress. The results suggest that Ymr134wp is essential to aerobic growth due to its function in ergosterol biosynthesis, playing a role in maintaining mitochondrial and plasma membrane integrity and consequently impacting the iron homeostasis, respiratory metabolism and antioxidant response.

1. Introduction

In 1997, applying a random mutagenesis approach, Babcock et al. isolated a mutant that was named *bm-8*, which was unable to grow under low-iron-containing media. The expression of two genes, *YFH1* or *YMR134W,* in the *bm-8* background resulted in the complementation of the mutant phenotype back to the wild-type state. The group described that the gene responsible for allelic complementation of the low iron growth defect was *YMR134W*; however, they focused their studies on the *YFH1* gene (able to restore the wild-type state of *bm-8* only when over-expressed) because of its homology to the human frataxin gene [1]. Later on, several studies confirmed the hypothesis that yeast frataxin (Yfh1p) is a mitochondrial protein involved in iron homeostasis and it is now accepted as one of the components of the mitochondrial machinery involved in [Fe–S] cluster synthesis, acting either as an iron donor or as a chaperone interacting with desulfurase/scaffold proteins involved in cluster [Fe–S] assembly [2]. In contrast, the other gene, *YMR134W,* was not further studied, but the *bm-8* mutant phenotype suggested that *YMR134W* may also be involved in iron metabolism.

Previously, genome-wide analyses produced more data about *YMR134W*: Ymr134wp is localized at the endoplasmic reticulum (ER) [3] and it is involved in mitochondria morphogenesis [4]. Later on, Snoek and Steensma showed that the gene *YMR134W* is essential when yeast is grown under aerobic conditions, but not in anaerobic ones [5]. Finally, Jerry Kaplan (2007), in a communication posted at the *Saccharomyces* Genome Database, renamed the gene *YMR134W* as *ERG29,* explaining that "Erg29p (*YMR134W* product) binds to Erg25p and affects its activity; *erg29* null mutant is non-viable in a respiratory competent background but is viable in a respiratory-defective background; *erg29* mutation is synthetically lethal with *mmt1* and *mmt2* null mutations." [http://www.yeastgenome.org/cgi-bin/locusHistory.pl?dbid=S000004741]. Erg25p is a C-4 sterol methyl oxidase, responsible for catalyzing the conversion of 4,4-dimethylzymosterol to zymosterol, a precursor of ergosterol [6]. *MMT1* and *MMT2* are mitochondrial metal transporters [7], which further indicate the involvement of *YMR134W* in mitochondrial and iron metabolism. In the following year, Tarassov et al. in a genomic-wide study using a Protein-fragment

* Corresponding author. Address: Av. Lineu Prestes, 580, Bloco 16, Semi Industrial, primeiro andar, sala 135, Zip code: 05508-000, Cidade Universitária Armando Salles de Oliveira, São Paulo – SP, Brazil. Fax: +55 11 38156386.
E-mail addresses: almeidam.gabriel@gmail.com (G. Moretti-Almeida), nettoles@ib.usp.br (L.E.S. Netto), smgisele@usp.br (G. Monteiro).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.07.024

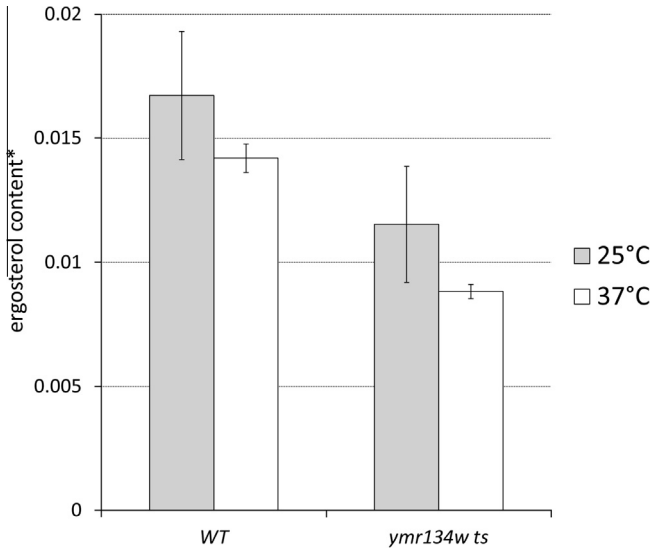

Fig. 1. Ymr134wp is involved in ergosterol biosynthesis. Total ergosterol was extracted and quantified using the Sterol Quantitation Method (SQM) exactly as described by Arthington-Skaggs et al. (1999). *Expressed as a percentage of the wet weight of the cell (grown on YPD media). The graph represents the average and standard deviation (±S.D.) from three independent experiments. The difference between mutant and wild-type was statistically significant for p-value < 0.01 (determined from paired t test) at both temperatures.

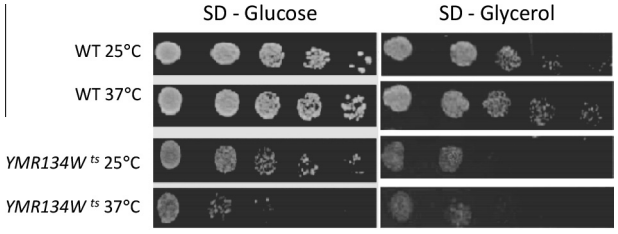

Fig. 2. *YMR134W*^{ts} cells have impaired growth on a respiratory carbon source (glycerol). SD-URA$^-$ plates with indicated carbon source. The first spot on the left in each lane corresponds to 5 μl of the cell suspension at $A_{600\ nm}$ = 0.2. Four serial 1:5 dilutions were performed from left to right. Figure representative of at least three independent experiments.

Complementation Assay, also observed a physical interaction between Erg25p and Ymr134wp [8], reinforcing the suggestion that the last protein could be involved in ergosterol synthesis.

Ergosterol is a major lipid component in yeast membranes that is associated with structural features such as fluidity and permeability, which are relevant for proper functioning of membrane proteins. Several steps in the ergosterol biosynthetic pathway are essential to yeast cell viability, and because of this, the major class of antifungal—the azoles—target their components [9]. Ergosterol and iron have a strict relationship because several key enzymes in ergosterol biosynthesis are iron-centered proteins. In particular, Erg25p is an oxo-diiron enzyme, essential to aerobic growth of yeast [6]. In addition, ergosterol synthesis is strictly dependent on aerobic growth conditions because twelve oxygen molecules are required for this process [10].

Seventeen years after the completion of the yeast genome sequence [11], more than 1600 genes do not have a function clearly assigned. One of them is *YMR134W* which is annotated as uncharacterized. In the present study, we took advantage of a thermosensitive strain (*YMR134W*^{ts}) to study the role of *YMR134W*, an essential gene in yeast. Here we show that Ymr134wp is involved in ergosterol biosynthesis and its decreased activity in a thermosensitive strain leads to the formation of functionally impaired mitochondria affecting the growth of yeast in a respiratory carbon source (such as glycerol) and the oxygen consumption by the cells. The mutation also increased the iron uptake of the cells from the culture media, but it diminished the concentration of iron inside the mitochondria. Besides, *YMR134W*^{ts} cells were more sensitive to oxidative stress and presented increased catalase activity. Together, our data suggest that the phenotypes relating *YMR134W* to iron metabolism and mitochondria morphology and function are due to its role in ergosterol biosynthesis.

2. Materials and methods

2.1. Materials

2.1.1. Thermosensitive strain YMR134W^{ts} genotype

(*MATa, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ0, met15Δ0, can1Δ::LEU2, MFA1pr::His3, ymr134w-ts::URA3*). The thermosensitive collection was kindly provided by Shay Ben-Aroya and Philip Hieter from the University of British Columbia, Canada [12]. The wild-type strain BY4741 (*MATa, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0*) was purchased from Invitrogen (Invitrogen, Carlsbad, CA, USA) and it was transformed with empty vector pYES.2.1 TOPO-TA (Invitrogen) to confer to the wild-type strain the capacity to grow on media without uracil, such as *YMR134W*^{ts} cells.

2.1.2. Culture media

YPD (yeast extract 1%, peptone 2% and dextrose 2%), YEPG (yeast extract 1%, ethanol 2%, peptone 2% and glycerol 2%), YPGAL (yeast extract 1%, peptone 2% and galactose 2%) or SD-URA$^-$ (Yeast Nitrogen Base without amino acids 0.7% + ammonium sulfate 0.5%, glucose 2% or glycerol 2%, DROPOUT 0.12% (mix of all amino acids, except leucine, uracil, tryptophan, and histidine), histidine 0.8%, leucine 0.8%, tryptophan 0.8%. Agar (2%) was added to solid media.

Hydrogen peroxide was obtained from Merck (Darmstadt, Germany); *tert*-butyl hydroperoxide and most of other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.1.3. Viability assay conditions

YMR134W^{ts} and wild-type cells growth analyses on different carbon sources or exposed to oxidative agents. Cells were initially inoculated in a 5 ml SD-glucose URA$^-$ at 25 °C, overnight, at 180 rpm (Innova 42R NEW BRUNSWICK SCIENTIFIC, Eppendorf, Hamburg, Germany). Then cells were diluted on 50 ml fresh media (YPD, YEPG or SD-glucose URA$^-$, specifically indicated in the figure legends) at an optical density ($OD_{600\ nm}$) equal to 0.2 and then incubated in the permissive temperature (25 °C) for 4 h. The cells were then divided into two equal aliquots and incubated further at 25 °C or at non-permissive temperature (37 °C) overnight (precisely 16 h). The $OD_{600\ nm}$ was corrected to 0.2 in sterile water and an additional four serial dilutions of fivefold were carried out. An aliquot of 5 μl of each cell dilution was dropped into solid SD-URA$^-$ media (glucose 2% or glycerol 2% + glucose 0.1% as carbon source) containing or not (control) hydrogen peroxide (H_2O_2 0.5 mM) or *tert*-butyl hydroperoxide (0.75 mM). The plates were incubated at permissive temperature for 3 days and photographed (Major Science-UVDI-312; Saratoga, CA, USA).

2.1.4. Quantitation of ergosterol

The amount of ergosterol in the cells was spectrophotometrically determined, exactly as described by Arthington-Skaggs et al. The method takes advantage of the unique spectral absorption pattern produced by sterols in the range 240–300 nm [13].

2.1.5. Cellular iron uptake measurement

Cells were grown as described above in YPD media. The total iron of the media was obtained from YPD before cell inoculation. The iron content was measured colorimetrically using a TPTZ re-

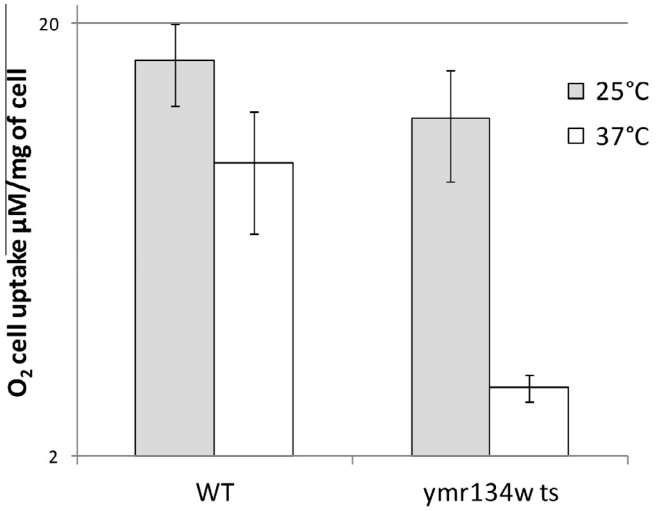

Fig. 3. Impaired oxygen consumption in *YMR134W*^{ts} cells. Cells were grown on YEPG media for 16 h harvested by centrifugation, resuspended in buffer containing sucrose/ethanol as respiratory substrates, and the oxygen uptake was measured using Clark electrodes for at least 30 min. The graph represents the average and standard deviation (±S.D.) from three independent experiments. The difference between mutant and wild-type was statistically significant for *p*-value <0.01 (determined from paired *t* test) at non-permissive temperature.

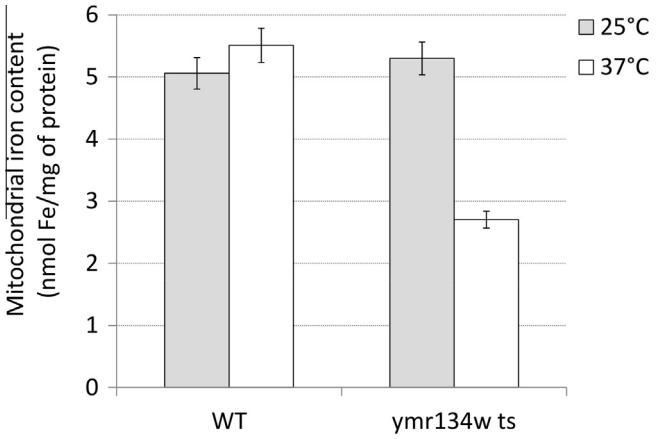

Fig. 4. *YMR134W*^{ts} cells possess low iron concentration in their mitochondria at non-permissive temperature. Cells were grown on YPGAL to avoid glucose repression in mitochondria proliferation. The graph represents the average and standard deviation (±S.D.) from three independent experiments. The difference between mutant and wild-type was statistically significant for *p*-value <0.01 (determined from paired *t* test) only at non-permissive temperature.

agent (2,4,6-tri(2-pyridyl)-1,3,5-triazine) which strongly binds reduced iron and this complex possesses an extinction coefficient of 21.600 M^{-1} cm^{-1} at 593 nm (Hach, Loveland, CO, USA). Detection limits are from 0.01 to 1.80 mg/L. The components of the kit include ascorbic acid in high concentration to reduce iron content, so the values represent total iron (reduced and oxidized). After growth, cells were harvested and supernatant (culture media) was used to measure the remaining iron content. A standard curve with known concentrations of $FeCl_3$ was used to calculate iron concentrations of the samples.

2.1.6. Cellular iron content measurement

Cells were grown as described above in YPD media and collected by centrifugation after 16 h at permissive or non-permissive temperature. The cell pellets were washed twice in distillated cold water and were disrupted in 1 volume of glass beads and 2 volumes of 3.5% sulfosalicylic acid. The suspension was vortexed for 20 min at 4 °C in a multifold vortex and harvested at 16,000 g. This procedure was repeated twice and the supernatants were combined. The pH of the samples was adjusted to pH 4.0 with NaOH, because TPTZ reagent only produces color between pH 3.0–5.0. Aliquots of 50 µl were used to react with TPTZ, as described above. The pellets of disrupted cells were homogenized with Tris–HCl pH 7.5 buffered guanidine 5 M overnight and an aliquot of each sample was used to measure total protein content. Protein concentration was determined with the Bradford reagent from BIORAD (Hercules, CA, USA), using bovine serum albumin (Sigma) as a standard.

2.1.7. Mitochondrial iron content

The mitochondrially enriched fraction was obtained from cells grown on YPGAL, and following the protocol exactly as described earlier [14]. The mitochondria were then lysed in sterile water and an aliquot possessing 200 µg of protein (previously determined with Bradford reagent) of this homogenate was used to measure the iron content as described above.

2.1.8. Oxygen uptake measurements

Experiments were carried out using a Clarke-type electrode in a glass cuvette equipped with magnetic stirring. To adjust the uptake for cell concentration, the cell dry weight was measured until three consecutive constant values. The cells were incubated at 30 °C in buffer containing HEPES 10 mM pH 7.2, sucrose 250 mM, KCl 65 mM and ethanol 2.5 mM for at least 30 min [14].

2.1.9. Catalase activity assay

Oxygen-release measurements were carried out using a Clarke-type electrode in a glass cuvette equipped with magnetic stirring. To measure oxygen release, corresponding to catalase activity, the cells (grown on YPD) were disrupted in a vortex using glass beads in phosphate buffer saline 1× with 1 mM of PMSF added to inhibit proteolysis. The protein extract was harvested at 16,000*g* for 30 min at 4 °C, the supernatant was collected and the total protein amount was quantified by Bradford assay [15]. Oxygen release due to catalase activity ($2H_2O_2 \rightarrow 2H_2O + O_2$) in cell extracts was specifically determined using a Clark electrode at 30 °C (Yellow Springs Instrument Co.). The saturating oxygen concentration, which corresponds to the full scale of the electrode, was taken to be 0.225 mM [15]. The initial peroxide concentration added was 1 mM in 50 mM HEPES and 100 mM NaCl. The activity observed was completely abolished when 1 mM azide (inhibitor of catalase) was added previously to peroxide in the reaction media.

3. Results and discussion

The data published about phenotypes (aberrant mitochondrial morphology [4] and inability to growth in iron deficient media [1]) and about protein physical interactions (with Erg25p [8], a key enzyme in ergosterol biosynthesis) lead us to hypothesize that Ymr134wp is involved in ergosterol biosynthesis in yeast. To test this hypothesis, the content of total ergosterol was measured in wild-type and *YMR134W*^{ts} cells (Fig. 1). *YMR134W*^{ts} cells showed almost 40% less ergosterol compared with the wild-type strain at non-permissive temperature, suggesting a significant importance of Ymr134wp to the synthesis of this lipid. Corroborating this idea, Ymr134wp is localized in the ER [3], where most of the components of the superpathway of ergosterol biosynthesis are present. In addition, ergosterol mutant strains and *ymr134w* mutant cells share several common phenotypes. Mutation of *erg25* (LT06) [6], *erg3* and *erg6* [16] renders cells unable to grow under low iron

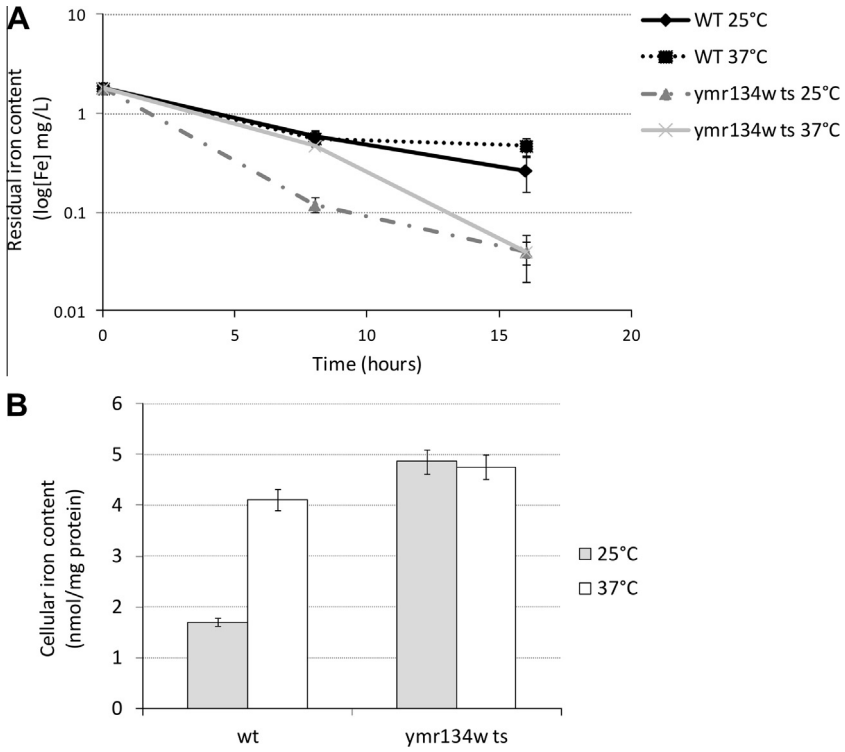

Fig. 5. Consumption and accumulation of iron is increased in *YMR134W*^{ts} cells. (A) The residual iron content on YPD media was measured at 0, 8 and 16 h after cell inoculation. The total initial iron content on YPD media was 1.8 mg/L. (B) Total cellular iron content was measured at 16 h after cell inoculation as described in "Section 2". The graphs represent the average and standard deviation (±S.D.) from three independent experiments. The difference between mutant and wild-type was statistically significant for *p*-value <0.01 (determined from paired *t* test) at both temperatures in (A) and only at permissive temperature in (B).

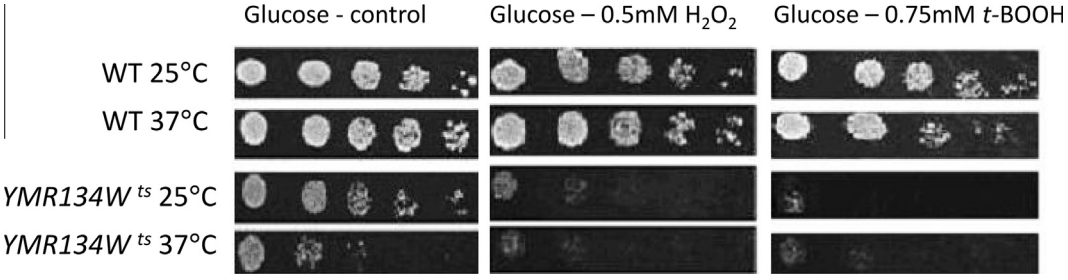

Fig. 6. *YMR134W*^{ts} cells are sensitive to oxidative stress. SD-URA$^-$ plates in the absence (control) or presence of the oxidant agents, hydrogen peroxide (H_2O_2), and *tert*-butyl hydroperoxide (*t*-BOOH). Serial dilution performed as described in the legend of Fig. 2. Figure representative of at least three independent experiments.

conditions, such as observed in the *ymr134w* (*bm-8*) mutant cells [1]. Strains whose expression of *YMR134W* or ergosterol biosynthetic genes was strongly inhibited by doxycycline addition (through the regulation of a $TetO_7$ promoter) produced aberrant mitochondrial morphology profile [4]. In this regard, the ergosterol deficiency observed in *YMR134W*^{ts} cells (Fig. 1) is in agreement with these previous results and it can explain them, implicating Ymr134wp as a key component of ergosterol biosynthesis.

Ymr134wp was firstly related to iron metabolism [1] and several studies have suggested the relationship between mitochondrial function (mainly oxygen consumption) and iron metabolism [17–21]. It is well accepted that heme (an iron cofactor synthesized into mitochondria) is the oxygen sensor molecule in yeast and that this pathway is mediated by the Heme Activator Protein (Hap1p) [22]. In particular, Hap1p induces the expression of *ROX1* repressor that, in turn, inhibits the expression of the ergosterol import system from extracellular media in the presence of oxygen [23]. So, ergosterol supplementation under aerobic conditions is not effective in reverting phenotype of mutant genes involved in ergosterol biosynthesis because the absence of its importer system. However, the supplementation of media with ergosterol under anaerobic conditions renders reversal of lethal phenotype of *Δymr134w* [5] what reinforce our data involving this gene in ergosterol biosynthesis. Besides, the ergosterol biosynthesis links iron and oxygen cell-requirements because this anabolic pathway requires several iron-dependent enzymes (heme and oxo-diiron) and twelve oxygen molecules as substrate to generate ergosterol [10]. Remarkably, it has been suggested that the ability of organisms, such as *S. cerevisiae*, to grow aerobically or anaerobically is due to the presence of sterol importers that are only active in the absence of oxygen [5].

Since depletion of any gene involved in ergosterol biosynthetic pathway leads to mitochondrial morphology aberration [4], it would be expected that *YMR134W*^{ts} cells possessed impaired mitochondrial function and deficient growth in a respiratory carbon source, such as glycerol. In fact, *YMR134W*^{ts} cells grew poorly in the presence of this carbon source (Fig. 2). In addition, oxygen consumption was also analyzed to assess mitochondrial function and a

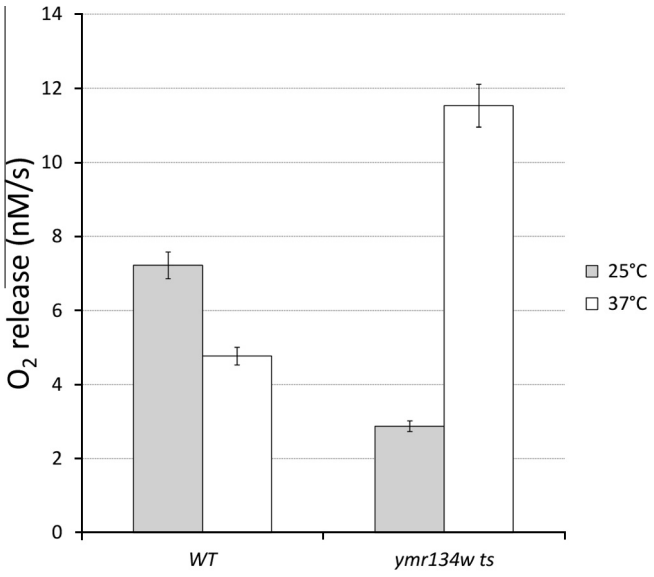

Fig. 7. *YMR134W*^{ts} cells are in oxidative stress. Cells were grown on YPD. Catalase activity measured by the release of molecular oxygen, one of the reaction products. The graph represents the average and standard deviation (±S.D.) from three independent experiments. The difference between mutant and wild-type was statistically significant for *p*-value <0.01 (determined from paired *t* test) at both temperatures.

considerable decrease in oxygen uptake was observed at the non-permissive temperature in *YMR134W*^{ts} cells (Fig. 3), suggesting that the mitochondrial function was affected.

Due to the ability of *YFH1* gene overexpression to rescue *bm-8* phenotype to wild-type state, it was expected that *YMR134W*^{ts} cells would present similar phenotype in respect to the mitochondrial iron homeostasis. *yfh1* mutant cells accumulate high amounts of iron into mitochondria compartment, but this metal is not available to biological processes, such as [Fe–S] cluster biogenesis, because it is precipitated with phosphate [21]. Unexpectedly, *YMR134W*^{ts} cells at the non-permissive temperature possessed almost half of the concentration of iron in the mitochondria (Fig. 4). Therefore, in this aspect, inhibition of the function of the *YMR134W* and the *YFH1* genes resulted in opposite outcomes (depletion or accumulation of mitochondrial iron content, respectively). Although the overexpression of Yfh1p was able to complement *bm-8* growth defects, this protein has no relation to ergosterol biosynthesis, since the levels of ergosterol in *yfh1* mutants are normal [24]. In contrast to Yfh1p, Ymr134wp is not localized in mitochondria but in the ER [3], features which taken together could be related to these distinct phenotypes.

Next, the ability of *YMR134W*^{ts} cells to uptake iron from the culture medium and accumulate this metal inside the cell was investigated. Remarkably, *YMR134W*^{ts} cells consumed high amounts of iron from the culture media and accumulate it (Fig. 5). After 16 h of growth at permissive or non-permissive temperatures, *YMR134W*^{ts} cells almost completely exhausted the iron content of the media (Fig. 5A). Interesting, in wild-type strain the non-permissive temperature increased the iron accumulation in the cells (Fig. 5B). At least to our knowledge, this phenomenon has not yet been reported to yeast, and deserves further studies. In any case, the data described in Fig. 5 suggests that *YMR134W*^{ts} cell iron uptake is deregulated and cells probably accumulate excessive iron in the cytosol due to the fact that delivery of iron to mitochondria in these cells is impaired (Fig. 4), suggesting a possible condition of oxidative stress [20,25].

Therefore, the response of *YMR134W*^{ts} cells to oxidative stress was investigated. Accordingly, *YMR134W*^{ts} cells presented high sensitivity not only to hydrogen but also to *tert*-butyl hydroperoxide insult (Fig. 6). In fact, at both temperatures (25 or 37 °C), *YMR134W*^{ts} cells suffered a significant decrease in their viability in the presence of peroxide. In addition, the catalase activity was measured in both strains, since this enzyme participates in the antioxidant response. *YMR134W*^{ts} cells displayed double the catalase activity at the non-permissive temperature when compared with wild-type (Fig. 7). However, these cells presented lower catalase activity at permissive temperature, which is in agreement with cell sensitivity to oxidant insult in this growth condition. It is noteworthy to observe that, also at the permissive temperature, *YMR134W*^{ts} cells consumed and accumulated excessive iron, explaining this sensitivity to hydroperoxides (Fig. 5).

In agreement with these results, Folmer et al. [26] showed that during adaptation to hydrogen peroxide the plasma membrane suffers modulation of their biophysical properties, resulting in decreased diffusion rate of hydrogen peroxide. Interestingly, this modulation was dependent on ergosterol biosynthesis, since *Δerg3* or *Δerg6* cells, displays increased permeability to hydrogen peroxide [26]. So, it is expected that in addition to excess of iron, *YMR134W*^{ts} cells are also more permeable to peroxides, both factors probably contributing to lower viability of this strain when exposed to these oxidants (Fig. 6). In addition, there is a relationship between the progression in the pathway from ergosterol precursors toward ergosterol and the ability of the lipid to protect membrane against oxidation, influencing the cell resistance to oxidative stress [27]. Considering that Ymr134wp is important to Erg25p function, the ergosterol pathway was disrupted early in *YMR134W*^{ts} cells, leading to formation of a less protective structural lipid against oxidative stress.

The data suggest that Ymr134wp is a key component in ergosterol biosynthesis, leading the mitochondria of *YMR134W*^{ts} cells to present problems in their membrane composition, probably resulting in deficiency of iron transport from the cytosol to the mitochondria. The critical transporters involved in mitochondrial iron import are still poorly understood. Among the probable proteins involved in this process are Mmt1p, Mmt2p and Mrs3p, Mrs4p. Mmt1p and Mmt2p (mitochondrial metal transporters) are putative mitochondrial iron transporters since their over-expression increases iron accumulation into mitochondria by 2- to 5-fold when compared to the wild-type [7]. Bioinformatics' predictions suggest that both Mmt1p and Mmt2p are integral to the membrane (TMHMM tool, an application available at The Center for Biological Sequence Analysis at the Technical University of Denmark DTU). However, the role of these transporters is controversial since the double mutant *Δmmt1Δmmt2* does not show decreased mitochondrial iron content [28]. The best candidates for mitochondrial iron importers are Mrs3 and Mrs4 proteins (''Mitochondrial RNA Splicing'') whose double deletion causes impairment in mitochondrial iron accumulation [28,29]. It is expected that mutations damaging ergosterol biosynthesis result in membrane fluidity alterations, impairing the proper function of integral membrane protein transporters. Consequently, mutations in iron transporters to the mitochondria in *ymr134w* mutant cells could cause a severe iron deficiency in the organelles of these cells, leading to lethality. This hypothesis is in agreement with the observation of Jerry Kaplan (2007) that ''*erg29* mutation is synthetically lethal with *mmt1* and *mmt2* null mutations''. The lower intra-mitochondrial iron concentration in *YMR134W*^{ts} cells (Fig. 5) could be related to the inappropriate function of mitochondrial iron transport process.

The relationship of the ergosterol content and the mitochondria profile is known for a long time (Jollow et al.). When cells are cultivated in anaerobic conditions and limiting the levels of the lipids (ergosterol, and unsaturated fatty acids) mitochondrial and vacuole morphology are profoundly altered [30]. In agreement with this data, the results presented here show that low ergosterol content

in *YMR134W*^{ts} cells probably affects mitochondrial membrane composition, resulting in low iron concentration inside the organelle, low oxygen consumption, and consequently less ability to grow in a respiratory carbon source. In consequence, the disturbed iron homeostasis and altered plasma membrane peroxide permeability prone cells to oxidative stress.

4. Conclusions

In conjunction, the data suggests the involvement of the *YMR134W* gene in the ergosterol biosynthesis, which affects mitochondrial and plasma membrane composition, and, in turn, has implications for iron homeostasis, mitochondria function, respiratory growth and antioxidant cell protection.

Acknowledgments

We thank Dr. Marilene Demasi for helpful comments and discussion of the results. We also thank to São Paulo Research Foundation FAPESP (Grant 2009/01,303-1) and CNPq for financial support.

References

[1] Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M. and Kaplan, J. (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. Science 276, 1709–1712.
[2] Li, H., Gakh, O., Smith 4th, D.Y. and Isaya, G. (2009) Oligomeric yeast frataxin drives assembly of core machinery for mitochondrial iron-sulfur cluster synthesis. J. Biol. Chem. 284, 21971–21980.
[3] Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S. and O'Shea, E.K. (2003) Global analysis of protein localization in budding yeast. Nature 425, 686–691.
[4] Altmann, K. and Westermann, B. (2005) Role of essential genes in mitochondrial morphogenesis in *Saccharomyces cerevisiae*. Mol. Biol. Cell 16, 5410–5417.
[5] Snoek, I.S. and Steensma, H.Y. (2006) Why does Kluyveromyces lactis not grow under anaerobic conditions? Comparison of essential anaerobic genes of *Saccharomyces cerevisiae* with the Kluyveromyces lactis genome. FEMS Yeast Res. 6, 393–403.
[6] Li, L. and Kaplan, J. (1996) Characterization of yeast methyl sterol oxidase (ERG25) and identification of a human homologue. J. Biol. Chem. 271, 16927–16933.
[7] Li, L. and Kaplan, J. (1997) Characterization of two homologous yeast genes that encode mitochondrial iron transporters. J. Biol. Chem. 272, 28485–28493.
[8] Tarassov, K., Messier, V., Landry, C.R., Radinovic, S. and Serna Molina, M.M. (2008) An in vivo map of the yeast protein interactome. Science 320, 1465–1470.
[9] Kristan, K. and Rizner, T.L. (2012) Steroid-transforming enzymes in fungi. J. Ster. Biochem. Mol. Biol. 129, 79–91.
[10] Maczek, J., Junne, S., Nowak, P. and Goetz, P. (2006) Metabolic flux analysis of the sterol pathway in the yeast *Saccharomyces cerevisiae*. Bioprocess Biosyst. Eng. 29, 241–252.
[11] Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., et al. (1996) Life with 6000 genes. Science 274, 563–567.
[12] Ben-Aroya, S., Coombes, C., Kwok, T., O'Donnell, K.A., Boeke, J.D. and Hieter, P. (2008) Toward a comprehensive temperature-sensitive mutant repository of the essential genes of *Saccharomyces cerevisiae*. Mol. Cell 30, 248–258.
[13] Arthington-Skaggs, B.A., Jradi, H., Desai, T. and Morrison, C.J. (1999) Quantitation of ergosterol content: novel method for determination of fluconazole susceptibility of *Candida albicans*. J. Clin. Microbiol. 37, 3332–3337.
[14] Monteiro, G., Kowaltowski, A.J., Barros, M.H. and Netto, L.E. (2004) Glutathione and thioredoxin peroxidases mediate susceptibility of yeast mitochondria to Ca(2+)-induced damage. Arch. Biochem. Biophys. 425, 14–24.
[15] Munhoz, D.C. and Netto, L.E. (2004) Cytosolic thioredoxin peroxidase I and II are important defenses of yeast against organic hydroperoxide insult: catalases and peroxiredoxins cooperate in the decomposition of H_2O_2 by yeast. J. Biol. Chem. 279, 35219–35227.
[16] Minear, S., O'Donnell, A.F., Ballew, A., Giaever, G., Nislow, C., Stearns, T. and Cyert, M.S. (2011) Curcumin inhibits growth of *Saccharomyces cerevisiae* through iron chelation. Eukaryot. Cell 10, 1574–1581.
[17] Li, L. and Kaplan, J. (2004) A mitochondrial-vacuolar signaling pathway in yeast that affects iron and copper metabolism. J. Biol. Chem. 279, 33653–33661.
[18] Hausmann, A., Samans, B., Lill, R. and Mühlenhoff, U. (2008) Cellular and mitochondrial remodeling upon defects in iron-sulfur protein biogenesis. J. Biol. Chem. 283, 8318–8330.
[19] Shakoury-Elizeh, M., Protchenko, O., Berger, A., Cox, J., Gable, K., Dunn, T.M., Prinz, W.A., Bard, M. and Philpott, C.C. (2010) Metabolic response to iron deficiency in *Saccharomyces cerevisiae*. J. Biol. Chem. 285, 14823–14833.
[20] Lin, H., Li, L., Jia, X., Ward, D.M. and Kaplan, J. (2011) Genetic and biochemical analysis of high iron toxicity in yeast: iron toxicity is due to the accumulation of cytosolic iron and occurs under both aerobic and anaerobic conditions. J. Biol. Chem. 286, 3851–3862.
[21] Seguin, A., Santos, R., Pain, D., Dancis, A., Camadro, J.M. and Lesuisse, E. (2011) Co-precipitation of phosphate and iron limits mitochondrial phosphate availability in *Saccharomyces cerevisiae* lacking the yeast frataxin homologue (YFH1). J. Biol. Chem. 286, 6071–6079.
[22] Kwast, K.E., Burke, P.V., Staahl, B.T. and Poyton, R.O. (1999) Oxygen sensing in yeast: evidence for the involvement of the respiratory chain in regulating the transcription of a subset of hypoxic genes. Proc. Natl. Acad. Sci. USA 96, 5446–5451.
[23] Crowley, J.H., Leak Jr, F.W., Shianna, K.V., Tove, S. and Parks, L.W. (1998) A mutation in a purported regulatory gene affects control of sterol uptake in *Saccharomyces cerevisiae*. J. Bacteriol. 180, 4177–4183.
[24] Chen, O.S., Crisp, R.J., Valachovic, M., Bard, M., Winge, D.R. and Kaplan, J. (2004) Transcription of the yeast iron regulon does not respond directly to iron but rather to iron-sulfur cluster biosynthesis. J. Biol. Chem. 279, 29513–29518.
[25] Chen, O.S., Hemenway, S. and Kaplan, J. (2002) Genetic analysis of iron citrate toxicity in yeast: implications for mammalian iron homeostasis. Proc. Natl. Acad. Sci. USA 99, 16922–16927.
[26] Folmer, V., Pedroso, N., Matias, A.C., Lopes, S.C., Antunes, F., Cyrne, L. and Marinho, H.S. (2008) H_2O_2 induces rapid biophysical and permeability changes in the plasma membrane of *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1778, 1141–1147.
[27] Dupont, S., Lemetais, G., Ferreira, T., Cayot, P., Gervais, P. and Beney, L. (2012) Ergosterol biosynthesis: a fungal pathway for life on land? Evolution 66, 2961–2968.
[28] Foury, F. and Roganti, T. (2002) Deletion of the mitochondrial carrier genes MRS3 and MRS4 suppresses mitochondrial iron accumulation in a yeast frataxin-deficient strain. J. Biol. Chem. 277, 24475–24483.
[29] Mühlenhoff, U., Stadler, J.A., Richhardt, N., Seubert, A., Eickhorst, T., Schweyen, R.J., Lill, R. and Wiesenberger, G. (2003) A specific role of the yeast mitochondrial carriers MRS3/4p in mitochondrial iron acquisition under iron-limiting conditions. J. Biol. Chem. 278, 40612–40620.
[30] Jollow, D., Kellerman, G.M. and Linnane, A.W. (1968) The biogenesis of mitochondria: 3. The lipid composition of aerobically and anaerobically grown *Saccharomyces cerevisiae* as related to the membrane systems of the cells. J. Cell Biol. 37, 221–230.